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Patterns of Rare and Abundant Marine Microbial Eukaryotes

Ramiro Logares[1, *], Stéphane Audic[2, 3], David Bass[4], Lucie Bittner[2, 3, 5], Christophe Boutte[2, 3], Richard Christen[6, 7], Jean-Michel Claverie[8], Johan Decelle[2, 3], John R. Dolan[9], Micah Dunthorn[5], Bente Edvardsen[10], Angélique Gobet[2, 3], Wiebe H.C.F. Kooistra[11], Frédéric Mahé[2, 3, 5], Fabrice Not[2, 3], Hiroyuki Ogata[8, 12], Jan Pawlowski[13], Massimo C. Pernice[1], Sarah Romac[2, 3], Kamran Shalchian-Tabrizi[10], Nathalie Simon[2, 3], Thorsten Stoeck[5], Sébastien Santini[8], Raffaele Siano[14], Patrick Wincker[15], Adriana Zingone[11], Thomas A. Richards[16], Colomban de Vargas[2, 3], Ramon Massana[1]

[1] Institut de Ciències del Mar (ICM), CSIC, Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain
[2] UPMC Paris 06, ADMM UMR 7144, Station Biologique de Roscoff, 29682 Roscoff, France
[3] CNRS, ADMM UMR 7144, Station Biologique de Roscoff, 29682 Roscoff, France
[4] Natural History Museum, Department of Life Sciences, Cromwell Road, London SW7 5BD, UK
[5] University of Kaiserslautern, Department of Ecology, 67663 Kaiserslautern, Germany
[6] CNRS, SAE UMR 7138, Parc Valrose BP71, 06108 Nice Cedex 02, France
[7] Université de Nice-Sophia Antipolis, SAE UMR 7138,Parc Valrose BP71, 06108 Nice Cedex 02, France
[8] CNRS, IGS UMR 7256, Aix-Marseille Université, 13288 Marseille, France
[9] CNRS, LOV UMR 7093, UPMC Paris 06, 06230 Villefranche-sur-Mer, France
[10] University of Oslo,Department of Biosciences, P.O. Box 1066 Blindern, 0316 Oslo, Norway
[11] Stazione Zoologica Anton Dohrn, Ecology and Evolution of Plankton, Villa Comunale 1, 80121 Naples, Italy
[12] Tokyo Institute of Technology, Education Academy of Computational Life Sciences, Tokyo 152-8552, Japan
[13] University of Geneva, Department of Genetics and Evolution, 1211 Geneva, Switzerland
[14] Ifremer, Centre de Brest, DYNECO/Pelagos BP70, 29280 Plouzané, France
[15] CEA, Genoscope, 2 Rue Gaston Crémieux, 91000 Evry, France
[16] University of Exeter, Biosciences, Geoffrey Pope Building, Exeter EX4 4QD, UK

*: Corresponding author : Ramiro Logares, email address : ramiro.logares@gmail.com

Abstract:

Background

Biological communities are normally composed of a few abundant and many rare species. This pattern is particularly prominent in microbial communities, in which most constituent taxa are usually extremely rare. Although abundant and rare subcommunities may present intrinsic characteristics that could be crucial for understanding community dynamics and ecosystem functioning, microbiologists normally do not differentiate between them. Here, we investigate abundant and rare subcommunities

of marine microbial eukaryotes, a crucial group of organisms that remains among the least-explored biodiversity components of the biosphere. We surveyed surface waters of six separate coastal locations in Europe, independently considering the picoplankton, nanoplankton, and microplankton/mesoplankton organismal size fractions.

Results

Deep Illumina sequencing of the 18S rRNA indicated that the abundant regional community was mostly structured by organismal size fraction, whereas the rare regional community was mainly structured by geographic origin. However, some abundant and rare taxa presented similar biogeography, pointing to spatiotemporal structure in the rare microeukaryote biosphere. Abundant and rare subcommunities presented regular proportions across samples, indicating similar species-abundance distributions despite taxonomic compositional variation. Several taxa were abundant in one location and rare in other locations, suggesting large oscillations in abundance. The substantial amount of metabolically active lineages found in the rare biosphere suggests that this subcommunity constitutes a diversity reservoir that can respond rapidly to environmental change.

Conclusions

We propose that marine planktonic microeukaryote assemblages incorporate dynamic and metabolically active abundant and rare subcommunities, with contrasting structuring patterns but fairly regular proportions, across space and time.

Highlights

► Regular proportions of abundant and rare microeukaryotes across space and time ► Contrasting structuring patterns in abundant and rare regional communities ► Considerable phylogenetic diversity in the rare microeukaryote biosphere ► Metabolically active taxa are present among rare planktonic microeukaryotes

INTRODUCTION

Microbes are the dominant form of life in the oceans, playing fundamental roles in ecosystem functioning and biogeochemical processes at local and global scales [1-4]. However, limited knowledge of their diversity and community structure across space and time [5, 6] hinders our understanding of the links between microbial life and ecosystem functioning [7]. During the last decade, technological progress in molecular ecology and environmental sequencing has substantially boosted our understanding of marine microbes, unveiling notable patterns of abundant and rare sub-communities [4, 8, 9], reminiscent of patterns observed in classical plant and animal ecology [10]. The recently discovered large amount of rare taxa in microbial communities is now referred as the "Rare Biosphere" [11], whose exploration is made feasible today by means of High-Throughput Sequencing (HTS) technologies [12].

Abundant and rare microbial sub-communities may have fundamentally different characteristics and ecological roles. For example, rare marine microbes are hypothesized to include ecologically redundant taxa that could increase in abundance following environmental perturbation or change, and maintain continuous ecosystem functioning [13]. Locally rare taxa can also act as seeds for seasonal succession or sporadic blooms. Conversely, the drastic decrease in abundance or even extinction of a globally abundant oceanic microbe with no ecologically comparable counterpart in the rare biosphere could have significant and unpredictable effects on the global ecosystem. Most of the studies to date that have differentiated between abundant and rare marine microbial sub-communities concern bacteria. Abundant bacteria contribute mostly to biomass, carbon flow and nutrient cycling, while the generally large numbers of rare bacteria contribute predominantly to species richness [9]. Different strategies are observed among rare bacteria, such as dormant or inactive taxa that grow exponentially

when the right conditions are met [8, 14-16] or taxa that seem to remain members of the rare biosphere [15, 17] even when they have high relative metabolic activity. Rare bacteria may perform crucial ecosystem functions [18] and some of them can be metabolically more active than abundant taxa in the same community [14, 15]. Both abundant and rare bacteria can present similar biogeographic patterns [17, 19] indicating similar community assembly mechanisms.

Compared to bacteria, we know even less about abundant and rare marine microbial eukaryote (protists) sub-communities and, overall, marine protists remain as one of the least explored features of natural biodiversity [20]. Recent studies using *454* pyrosequencing [12] recovered few dominant protist taxa and a large number of rare ones from specific marine and freshwater communities [21-25]. Little information is available regarding protist metabolic activity. A recent study in freshwater lakes comparing rRNA/rDNA ratios (a proxy of microbial activity) suggested that, in contrast to bacteria, dormancy does not play an important role in planktonic protist communities [14].

Here we explore fundamental patterns of rare and abundant marine-planktonic protistan sub-communities occurring along the European coastline, from the North Sea (Norway) to the Black Sea (Bulgaria) (Figure 1A). Using *Illumina* [12] and, to a more limited extent, *454* HTS platforms, we generated a large dataset of both 18S rRNA and rDNA tags based on total RNA and DNA extracts from three organismal size-fractions, the pico- (0.8-3μm), nano- (3-20μm) and micro/meso- (20-2,000μm) plankton [3, 26]. The wide geographical and organismal scales of this dataset, combined with ultra-deep sequencing, allowed us to address the following main questions: Are the relative proportions of abundant and rare protist sub-communities fluctuating across space and time? What structural and biogeographic patterns present these sub-communities? Do

locally abundant taxa tend to be regionally abundant? And, are there specific phylogenetic and activity patterns associated to abundant and/or rare marine protistan sub-communities? We found that abundant and rare assemblages present contrasting structuring patterns and phylogenetic characteristics, despite a remarkable consistency in their relative proportions across individual samples. Furthermore, rare sub-communities included a large number of predominantly active lineages that presented biogeography.

RESULTS

General patterns of richness and evenness

Unless stated otherwise, our results derive from the *Illumina* V9 18S rRNA tag dataset clustered into 95% similarity Operational Taxonomic Units (OTUs; Table S1) and prepared using RNA extracts. The 95% threshold was selected for all downstream analyses in order to minimize any inflation of diversity estimates [27] caused by remaining tags (if any) with misincorporated nucleotides. In a local community, we defined OTUs as "abundant" when they reached relative abundances above 1% of the tags and "rare" when their abundances were below 0.01%, following other studies in bacteria [9, 17] and protists [25]. In the regional community (combination of local communities), the thresholds for abundant or rare were >0.1% and <0.001% respectively. In addition, we tested a 97% OTU clustering threshold and comparable patterns regarding proportions of locally abundant and rare sub-communities were obtained (Table S2).

In total, ~72% of all OTUs were found only in a single size fraction, being either the pico- (0.8-3µm), nano- (3-20µm) or micro/meso- (20-2,000µm) plankton (Figure S1). Similarly, ~75% of the rare and ~62% of the abundant OTUs in the regional community were restricted to a single organismal size fraction. This indicates that our sea-water filtering protocol used to separate total plankton communities into three distinct organismal size fractions was effective.

In rarefaction analyses considering all reads (5.69×10^6) and samples from the regional community, richness (based on 95% similarity OTUs) approached saturation at ~9,000 OTUs (Figure S2). OTU-richness also approached saturation in most local communities (between 800 – 3,000 OTUs; Figure S2A). The highest richness was

observed in the nano-plankton (3-20μm; 4,786 OTUs after normalization), and the lowest in the micro/meso-plankton (20-2000μm; 2,941 OTUs; Table 1). Evenness was low in the regional community, within different size fractions, and in all studied local communities (Figures S3A,B & S4), with the majority of OTUs being rare and only a few abundant. In the regional community, considering both pooled and separate size fractions, abundant taxa were <3.5% of the total OTUs while rare taxa were >63.4% of the OTUs (Table 1). When considering pooled normalized size fractions, the percentage of total reads falling into rare OTUs in the regional community was 1.1% (20,000 reads), while the percentage of total reads falling into abundant OTUs was 80.7% (1,448,079 reads).

Overall, a total of 20 out of 23 analyzed samples fitted the log-normal model [10, 28] of Species Abundance Distribution (SAD) according to the Akaike's Information Criterion [29] (Figure S4). Assuming a log-normal distribution, we fitted our regional community data to the truncated Preston log-normal model [10, 30] (Figure S5A) and estimated that we recovered 64-67% of the OTUs in the European coastal region (Figure S5B). Therefore, even though our deep *Illumina* sequencing approach recovered the majority of OTUs from our sample-set, extra sampling effort is needed to recover the total richness of the studied area.

Community structure across space, time, and organismal size-fractions

The proportions of locally rare (<0.01%) and abundant (>1%) OTUs (by our definition) were relatively constant across communities (Figure 1B, Table S1), with ranges of 66.2 – 76.6% for rare OTUs and 0.9 – 2.7% for abundant ones (Figure 1B, Table S1). Reads corresponding to locally abundant OTUs represented on average 70.1% (SD=9.5) of the

dataset, while reads corresponding to rare OTUs represented on average 1.9% (SD=0.7) (Figure 1B).

β-diversity, as described by Bray-Curtis dissimilarities between samples, within rare and abundant regional communities (i.e. pooled rare or abundant sub-communities) showed a moderate but significant correlation when considering normalized OTUs from all size fractions together (Mantel test $r_{(abundant|rare)}$=0.73; p<0.001) as well as within the pico-plankton (Mantel $r_{(abundant \mid rare,\ 0.8\text{-}3)}$=0.69; p<0.05). The correlation was weaker, but still significant, in the nano- and micro/meso-plankton (Mantel $r_{(abundant \mid rare,\ 3\text{-}20)}$=0.44; $r_{(abundant \mid rare,\ 20\text{-}2000)}$=0.46; p<0.05). These correlations indicate that some abundant and rare taxa share similar biogeography. Yet, the abundant regional community was structured mostly by size fraction, as the OTU composition of abundant micro/meso-plankton was more similar among samples of this fraction than to any sample of the pico- and nano- plankton (Figure 2A). In contrast, the rare regional community was mostly structured by sampling site, with samples from different organismal size fractions but from the same site being normally more similar in OTU composition when compared to samples from other sites (Figure 2A). Network analyses provided further insight into these patterns by showing that within the abundant regional community, the smaller size fractions (pico- and nano-plankton) shared more OTUs between them than with the larger size fraction (micro/meso-plankton; Figure 2B). In contrast, the rare regional community network showed that several OTUs were unique to single samples, and that the few shared OTUs tended to be shared between samples of the same site (Figure 2B).

Further exploration across samples of OTUs that were locally abundant in at least one sample (total 175 OTUs) showed that none of them presented abundances >1% in all samples. In analyses of individual size fractions, most OTUs with

abundances >1% were abundant at a single site/sample (Figure 3A-C), being often rare or of intermediate abundance elsewhere. Only one OTU within the fraction 3-20μm displayed abundances >1% in all samples (Figure 3B).

Phylogenetic patterning of abundant versus rare regional communities

The constructed phylogeny contained 11 reference OTUs that exclusively represented regionally abundant OTUs, 107 reference OTUs that represented both regionally abundant and rare OTUs, and 1,225 reference OTUs that exclusively represented regionally rare OTUs (Figure 4). While the majority of the regionally abundant OTUs had relatively close evolutionary relatives among the rare, the majority of the regionally rare OTUs had no close evolutionary relatives among the abundant. BLASTing *Illumina* representative reads from abundant and rare OTUs against each other supported this pattern. About 90% of the abundant OTUs (n=154) produced significant BLAST hits against the rare (that is, hits with coverage >97% and identity >70%), while only about 31% of the rare OTUs (n=5,329) produced significant hits against the abundant. Faith's Phylogenetic Diversity (PD) measure [31] was higher in the rare regional community when compared with the abundant at a similar sampling depth (Figure 4B). Both the Mean Phylogenetic Distance (MPD) and the Mean Nearest Taxon Distance (MNTD) [32] indicated that regionally abundant OTUs included in the phylogeny (n=118) clustered together at a higher frequency than what was expected by chance (Figure 4C). Such a pattern is expected to occur when the environment selects related taxa that share favorable traits [32, 33]. Conversely, the MPD and MNTD among regionally rare OTUs (n=1,332) did not present deviations from a random distribution (Figure 4C).

Activity versus abundance

In order to check to what extent the community and phylogenetic patterns described above are due to the use of RNA, and not DNA tags, and thus be partially explained by cellular activity, we analyzed 15 samples for which both DNA- and RNA-based tags (V4 18S, *454* tags) were obtained. The relative abundance of OTUs in the regional community that were present in both the DNA and RNA datasets showed on average a nearly 1:1 relationship (Figure 5A). Both the DNA and RNA recovered a number of OTUs as consistently rare or abundant in the regional community (Figure 5A). However, some OTUs were rare in the regional community according to the DNA dataset but showed intermediate abundances within the RNA dataset and vice versa. Approximately 25 OTUs were disproportionately underrepresented by RNA tags (Figure 5B, grey area), suggesting low-activity or large numbers of rDNA copies in the genome. On the contrary, ~20 OTUs were disproportionately overrepresented by RNA tags, which may result from high ribosomal activity (Figure 5B, yellow area).

DISCUSSION

Marine microbial eukaryotes constitute arguably the most poorly characterized biodiversity component in the biosphere [20]. Here we provide new insights into the structural and phylogenetic organization of their communities using the first ultra-deep sequencing dataset of 18S rRNA tags extracted from surface pico-, nano- and micro/meso-plankton collected at six marine-coastal locations across Europe. *Illumina* sequencing of >150 million V9 rRNA amplicons followed by highly-stringent sequence quality filtering allowed us to approach richness saturation (OTUs 95%) in both the entire regional community as well as in most local communities, therefore allowing the exploration of the rare protist biosphere. The highest richness was observed in the proximity of the smallest cell(body)-sizes (in the nano-plankton (3-20μm)), thus resembling patterns observed in animals by early ecologists [34, 35]. We estimate a recovery of ~64% of the total number of OTUs in the entire region, therefore more samples are required to cover the total diversity of European coastal waters. As observed in aquatic prokaryotes [9], most of the recovered OTUs (>63.0%) belonged to the rare biosphere; since we used RNA as template, we can attest that these OTUs represent living, ribosomically active cells.

Despite the strong spatio-temporal variability characterizing marine coastal waters and the different β-diversity among sites, the proportion of locally rare and abundant taxa was remarkably constant across all sampled communities. This pattern suggests community self-organization arising from local species interactions, with the observed regular proportions representing stable community configurations [36]. Given this striking consistency observed in our data, we hypothesize that in other marine planktonic communities >70% of protist OTUs are rare as well. Yet, it should be considered that rarity was analyzed according to one pre-existing definition; future

studies should explore multiple definitions in order determine which one is the most meaningful [10].

Both the abundant and rare regional communities demonstrated contrasting patterns regarding their general structure. The abundant regional community was predominantly structured by organismal size fraction, while the rare regional community was structured mostly according to geographic site. On the one hand, size fraction structuring reflects the fact that, excluding protists with complex cell cycles, ontogenic processes, or cell shapes and colony forms markedly distinct from a sphere, most taxa have rather constant cell sizes. On the other hand, site-associated clustering indicates that the differences between communities from different sites are larger than the differences between size fractions within the same site. Such groupings of the rare pico-, nano- and micro/meso-plankton were generated by only a few OTUs that were present in only one site and shared between the large and smaller size-fractions. These OTUs could represent low abundance protists with life cycles that involve different cell sizes, different lifestyles (host associated or not), as well as issues related to a non-optimal size fractionation during filtering. Site-associated clustering can be promoted by historical contingencies occurring in different communities, such as local random extinctions or stochastic immigration events [37], which are expected to have a larger impact in rare sub-communities, making them generally more distinct between each other than abundant counterparts.

Even though abundant and rare regional communities presented a markedly different general structure, we found a moderate but significant correlation in their β-diversity that points to similar biogeography for some rare and abundant taxa. This suggests that similar structuring processes can affect both abundant and rare sub-communities, and that the rare protist biosphere is not a random collection of taxa.

Comparable results have been reported for marine and lacustrine prokaryotes [17, 19, 38].

Underlying the β-diversity patterns at the regional level, locally abundant (>1%) OTUs within the pico-, nano-, and micro/meso-plankton showed marked variations in relative abundance between samples. Most locally abundant OTUs were abundant in only one sample, having intermediate or low (<0.01%) abundances in the others. This pattern, besides reflecting strong fluctuations in protistan abundance across heterogeneous coastal locations or seasonality in the same site [23, 39], points to a general decoupling between local and regional abundances, as most OTUs that are abundant in only one location will not be regionally abundant.

Last but not least, the rare protistan biosphere presented a distinctive phylogenetic composition, with a significant proportion of rare OTUs phylogenetically unrelated to abundant ones. In particular, several clades contained exclusively rare OTUs that were relatively distantly related in phylogenetic terms to the nearest abundant taxon. Although we cannot exclude that some taxa from these exclusively rare clades may be abundant in other locations/seasons, the overall pattern suggests that permanent or semi-permanent rarity (achieved e.g. through a low cell division rate) may be an evolutionary trait of some marine protist groups. Avoidance of competition, predation and parasitism are potential advantages of a low-abundance life [8], which could evolve through negative frequency-dependent selection [40]. On the contrary, rare OTUs in the regional community that were phylogenetically closely related to abundant ones could represent intra-genomic variation or erroneous variants of abundant OTUs generated during PCR or sequencing [41, 42], although we minimized this bias by working with a relatively loose definition of OTUs at 95% similarity threshold [27]. The structuring of the abundant regional community seems to have been influenced by

environmental selection of evolutionary related taxa presenting favorable traits, as abundant taxa were phylogenetically more closely related than expected by chance [32, 33]. Our comparison of rRNA- versus rDNA-derived OTUs indicated that both types of markers are broadly positively correlated, supporting the hypothesis that low metabolic activity or dormancy is not common among planktonic microbial eukaryotes [14, 43]. Thus, metabolically active taxa likely prevail in the protistan rare planktonic biosphere. In addition, rRNA/rDNA comparisons suggested that a disproportionately high activity is unusual in planktonic protists. Altogether, this contrasts markedly with planktonic bacteria, where dormancy appears to be more prevalent [9, 14] and where, for some taxa, activity can increase as abundance decreases [15].

CONCLUSION

Overall, our results indicate that marine-planktonic protist communities are composed of, predominantly active, abundant and rare sub-communities with contrasting structuring patterns and phylogenetic characteristics, which nevertheless display striking consistency in their local relative proportions, even in dissimilar coastal waters. Further analyses of protist community structuring in contrasting oceanic biomes will provide a wider test of the patterns we found in European coastal waters, contributing altogether to a better understanding of the community organization mechanisms in microbial eukaryotes and their links to local and global ecosystem functioning.

EXPERIMENTAL PROCEDURES

Sampling and *Illumina* / *454* sequencing

Surface (<5m depth) seawater samples were collected in six European coastal offshore sites: Blanes (Mediterranean), Gijon (Bay of Biscay), Naples (Mediterranean), Oslo (North Sea / Skagerrak), Roscoff (English Channel), and Varna (Black Sea) (Figure 1A, Table S3). Pico- (0.8-3µm) and nano- (3-20µm) plankton samples were collected using Niskin bottles. A total of 15 to 40 liters of water were pre-filtered through a 20µm sieve and then sequentially filtered through a polycarbonate membrane of 3 and 0.8µm. Micro/meso (20-2000µm) plankton samples were collected and concentrated using a 20µm-porosity plankton net during 20 min, then pre-filtered through a 2,000µm sieve and afterwards filtered through a 20µm polycarbonate membrane. Total RNA and DNA were extracted simultaneously from the three membranes. For *Illumina GAIIx* sequencing, hypervariable V9 18S tags were PCR amplified from cDNA obtained from RNA template, while V4 18S tags were PCR amplified from both DNA and RNA (cDNA) templates for *454-Titanium* sequencing.

Sequence analysis for *Illumina* reads

A total of 23 samples were selected for downstream analyses (Table S1). For the forward reads (hereafter "reads"), about 15 Gigabases of raw sequence data (100bp reads) were produced (Table S1). Reads (minimum 90bp) were quality-checked using a sliding 10bp-window and each window had to have a phred-quality average >34 to pass the control. The number of clean reads after quality control is shown in Table S1. Quality-checked reads were analyzed in QIIME v1.4 (Quantitative Insight Into Microbial Ecology; [44]). Reads were clustered into OTUs using UCLUST v1.2.22 [45] with a 95% similarity threshold. Chimeras were detected using ChimeraSlayer [46] with

a reference database derived from PR2 [47]. Taxonomy assignment was done by BLASTing [48] the most abundant (representative) sequence of each OTU against different reference databases, and unwanted OTUs (e.g. Metazoa and prokaryotes) were removed. The final curated *Illumina* RNA dataset included 5,696,049 reads. *Illumina* sequences are publicly available at MG-RAST (http://metagenomics.anl.gov/, accessions: 4549916.3 - 4549968.3).

Sequence Analysis for *454* reads

We analyzed 15 samples for which both DNA and RNA V4 18S tags were sequenced (Table S4); these samples were also present in the *Illumina* dataset (Table S1). All *454* reads between 200-500bp were run through QIIME v1.4. Reads were checked for quality using a sliding window of 50bp (Phred average >25 in each window) and truncated to the last good window. Sequences were denoised using DeNoiser (v 0.851; [49]) as implemented in QIIME v1.4 and then clustered into OTUs using UCLUST v1.2.22 with a 99% similarity threshold. Chimera detection and taxonomy assignment were done using the same approaches as with the *Illumina* reads. In the final V4 curated dataset, RNA included 233,085 reads and DNA 221,898 reads (total 454,983 reads). *454* sequences are publicly available at MG-RAST (http://metagenomics.anl.gov/, accessions: 4549916.3 - 4549968.3).

Final OTU tables

Single singletons as well as OTUs present in a single sample were removed from both *Illumina* V9 and *454* V4 OTU tables. For both datasets, we randomly subsampled OTU tables to the number of reads present in the sample with the lowest amount of reads.

This value was 78,000 reads per sample for *Illumina* and 3,000 reads per sample for *454*.

400

Ad hoc definitions of rare and abundant OTUs

OTUs were classified into abundant or rare in relation to their local and regional relative abundances. Locally abundant OTUs were defined as those with relative abundances >1%, and locally rare OTUs as those with abundances <0.01% following studies in
405 prokaryotes [9, 17] and protists [25]. Regional relative abundances for specific OTUs were calculated as the average of local relative abundances for such OTU across all samples, including zero values. The thresholds for defining abundant and rare at the regional level were arbitrarily defined as the local thresholds divided by a factor of 10. OTUs abundant in the regional community had a mean relative abundance >0.1%, while
410 regionally rare OTUs had a mean relative abundance <0.001%.

Diversity analyses

Most analyses were run in R [50] environment using the packages Vegan [51] and Picante [52]. Rarefactions and Species (OTUs) Accumulation Curves were calculated in
415 Vegan. OTU networks were constructed in QIIME based on the subsampled OTU table and graphically edited in Cytoscape [53] using the layout "Edge-Weighted Spring Embedded" with eweights.

Mapping of *Illumina* reads to reference Sanger sequences and phylogeny
420 construction

Representative reads of regionally abundant or rare OTUs were mapped separately using BLASTn to a custom V9 18S rDNA Sanger reference database based on the PR2

[47]. Using an e-value $<1x10^{-6}$ with a percentage of identity >90%, all abundant (n=154), and 95% of the rare (n=5,329) OTUs were assigned to reference taxa. The chosen parameters allowed for different OTUs to be mapped to the same Sanger reference taxa, and for this reason, the final phylogeny had less taxa than the sum of abundant and rare OTUs. For phylogeny construction, we extracted the full-length 18S sequence corresponding to all reference V9. Sequences were aligned using Mothur against the aligned SILVA 108 database (eukaryotes only). A Maximum Likelihood reference tree (8,311 sequences) was inferred using RAxML HPC-MPI (v7.2.8; [54]) under the model GTR+CAT/G+I and checked against other phylogenies of marine protists [55] for consistency. The tree was pruned using the R package APE (Analyses of Phylogenetics and Evolution; [56]) to keep only those reference taxa that were hit by abundant or rare OTUs. The final pruned tree, including 1,343 Sanger sequences, was used to calculate the Mean Phylogenetic Distance (MPD) and Mean Nearest Taxon Distance (MNTD) [32] with Picante. Phylogenetic diversity [31] was computed using Picante.

See more details on Experimental Procedures in Supplemental Information.

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FIGURE LEGENDS

Figure 1. Communities displayed regularity in the proportions of locally abundant and rare OTUs

(A) Sampled locations from the North Sea to the Black Sea.

(B) Percentage of locally abundant (>1%) and rare (<0.01%) OTUs as well as the corresponding *Illumina* reads across all samples, indicating different organismal size fractions (in μm) as well as geographic locations [in brackets] according to (A).

See also Tables S1, S2 & S3; Figures S1, S2 & S4.

Figure 2. Contrasting structuring patterns in abundant and rare regional communities

(A) UPGMA dendrograms based on Bray-Curtis dissimilarities between samples (normalized dataset) for both the rare and abundant regional communities. Branch colors indicate groups of samples originating from the same geographic site. Note that two large clusters are present within the abundant regional community, separating the pico- & nano- from the micro/meso-plankton.

(B) Networks representing abundant and rare regional communities. Larger nodes (circles) represent samples, while smaller nodes represent OTUs that may connect (that is, may be present in) different samples through edges (lines). The most relevant structuring features for both the abundant and rare regional communities were mapped onto the networks with colors. These were organismal size-fraction (abundant) and geographic origin (rare).

Figure 3. Locally abundant OTUs (>1%) tended to be abundant in a single sample

(A-C) Abundance across samples/sites of OTUs that were locally abundant in at least one sample, separated by size fractions. Heatmaps (left) indicate whether OTUs (vertical lines) were abundant (>1%; black), rare (<0.01% white) or had intermediate (grey) abundances in specific samples/locations. Histograms on the right indicate the number of samples in which OTUs were abundant in each dataset.

Figure 4. Phylogenetic patterns in the abundant and rare regional communities

(A) Maximum Likelihood phylogenetic tree based on reference Sanger 18S sequences (n=1,343) representing regionally abundant and rare OTUs (each branch of the tree derives from a reference sequence that represents an OTU). Red indicates sequences representing regionally abundant OTUs only, black points to sequences representing regionally rare OTUs exclusively, and green indicates sequences representing both regionally abundant and rare OTUs. The symbol "*" indicates some groups that were formed entirely by rare OTUs.

(B) Rarefaction analysis of Faith's Phylogenetic Diversity considering abundant and rare OTUs in the regional community.

(C) Mean Phylogenetic Distance (MPD) and Mean Nearest Taxon Distance (MNTD) estimates based on the phylogeny shown in (A). $MPD_{observed}$: observed MPD values; MPD_{null}: MPD values obtained from a null model. MPD_z: standardized effect size of MPD = $(MPD_{observed}-MPD_{null\ model})/sd(MPD_{null\ model})$; p = p-value. The same parameters are shown for the MNTD. Negative MPD_z & $MNTD_z$ values with $p<0.05$ indicate phylogenetic over-clustering.

Note that in (B) & (C), sequences that represented both abundant and rare OTUs ("shared") were considered within both the abundant and rare datasets.

Figure 5. Abundance of OTUs in the regional community according to rDNA and rRNA

(A) Average relative abundance of individual OTUs (dots) according to rRNA and rDNA. The abundance thresholds for abundant (>0.1%) and rare (<0.001%) are indicated with vertical and horizontal lines. The top-right grey corner indicates OTUs that were both abundant in RNA and DNA, while the bottom-left green corner indicates OTUs that were rare in both RNA and DNA. The yellow section indicates OTUs that were rare according to DNA and not rare according to RNA; the light blue section indicates the opposite. The best fitting linear regression is indicated, which was virtually identical to the 1:1 line.

(B) OTU rank-abundance curve based on rDNA (blue line) and the corresponding abundance for each OTU according to rRNA (red dots). Abundant (>0.1%) and rare (< 0.001%) thresholds are indicated with vertical lines. OTUs disproportionately over- or underrepresented in RNA in comparison to DNA are indicated in the yellow and grey areas respectively.

See also Table S4.

Figure 1

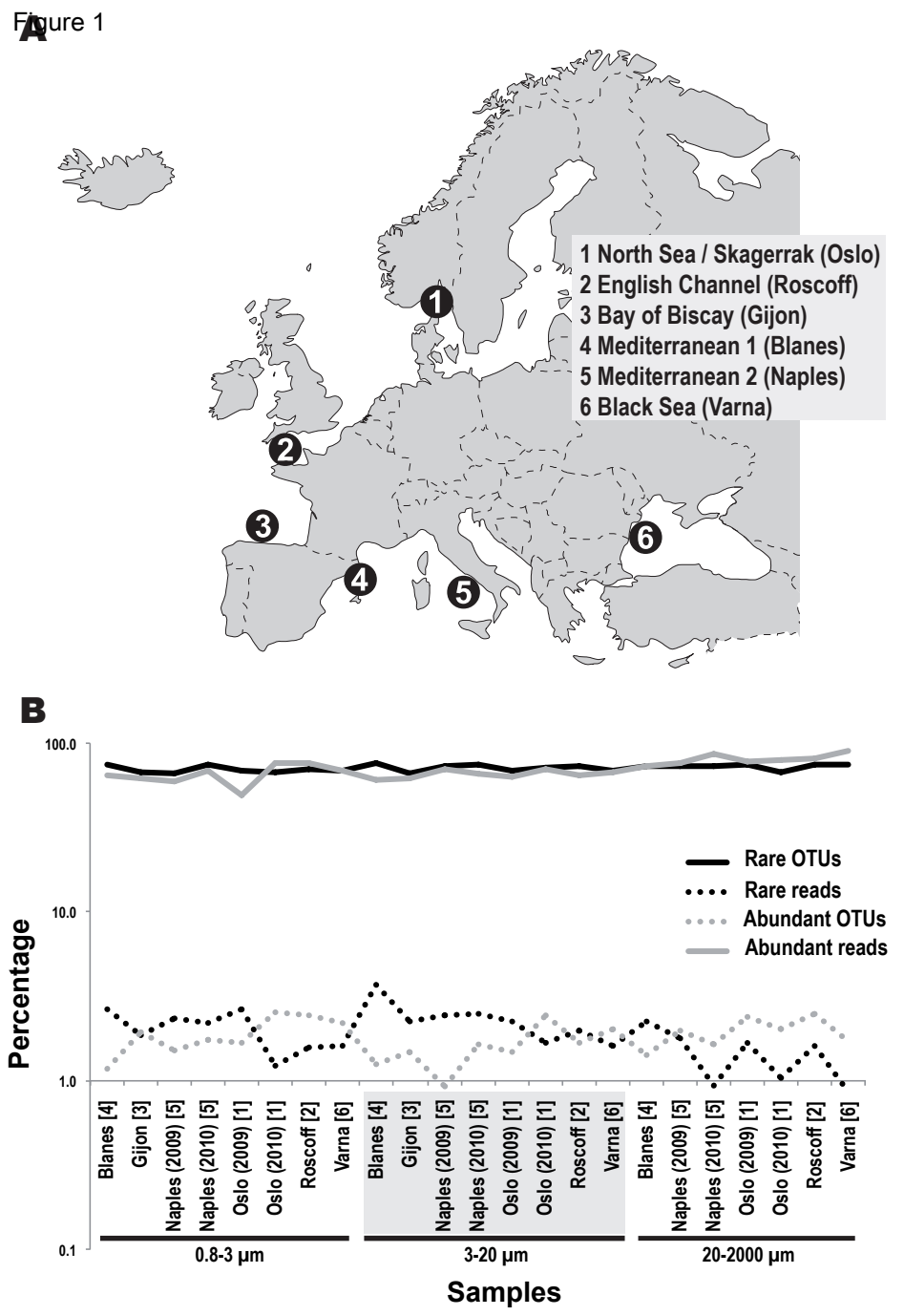

A
1 North Sea / Skagerrak (Oslo)
2 English Channel (Roscoff)
3 Bay of Biscay (Gijon)
4 Mediterranean 1 (Blanes)
5 Mediterranean 2 (Naples)
6 Black Sea (Varna)
B
100.0
10.0
1.0
0.1
Percentage
Rare OTUs
Rare reads
Abundant OTUs
Abundant reads
Blanes [4]
Gijon [3]
Naples (2009) [5]
Naples (2010) [5]
Oslo (2009) [1]
Oslo (2010) [1]
Roscoff [2]
Varna [6]
0.8-3 µm
3-20 µm
20-2000 µm
Samples

Figure 2

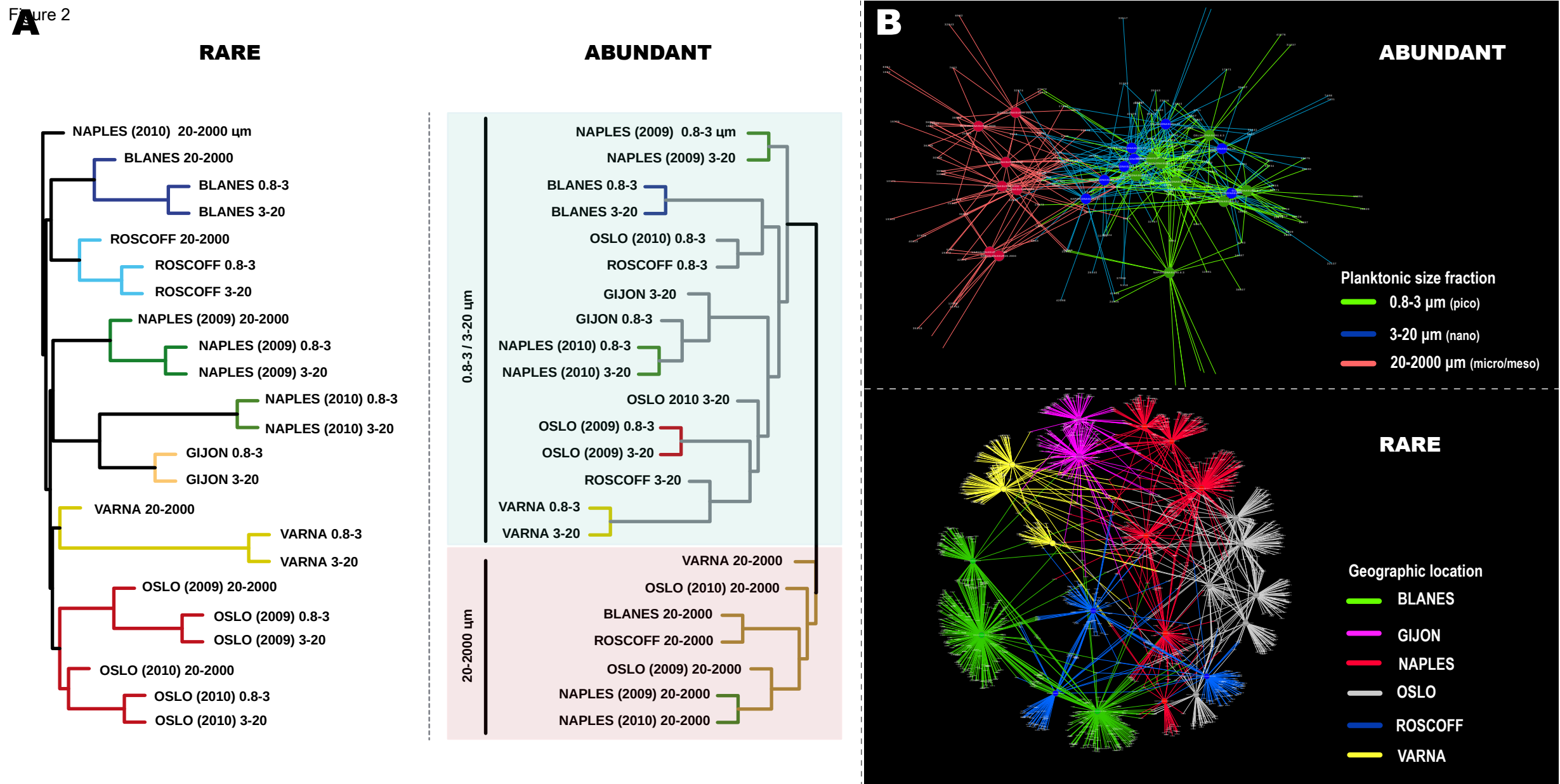

A
RARE
ABUNDANT
NAPLES (2010) 20-2000 µm
BLANES 20-2000
BLANES 0.8-3
BLANES 3-20
ROSCOFF 20-2000
ROSCOFF 0.8-3
ROSCOFF 3-20
NAPLES (2009) 20-2000
NAPLES (2009) 0.8-3
NAPLES (2009) 3-20
NAPLES (2010) 0.8-3
NAPLES (2010) 3-20
GIJON 0.8-3
GIJON 3-20
VARNA 20-2000
VARNA 0.8-3
VARNA 3-20
OSLO (2009) 20-2000
OSLO (2009) 0.8-3
OSLO (2009) 3-20
OSLO (2010) 20-2000
OSLO (2010) 0.8-3
OSLO (2010) 3-20
0.8-3 / 3-20 µm
NAPLES (2009) 0.8-3 µm
NAPLES (2009) 3-20
BLANES 0.8-3
BLANES 3-20
OSLO (2010) 0.8-3
ROSCOFF 0.8-3
GIJON 3-20
GIJON 0.8-3
NAPLES (2010) 0.8-3
NAPLES (2010) 3-20
OSLO 2010 3-20
OSLO (2009) 0.8-3
OSLO (2009) 3-20
ROSCOFF 3-20
VARNA 0.8-3
VARNA 3-20
20-2000 µm
VARNA 20-2000
OSLO (2010) 20-2000
BLANES 20-2000
ROSCOFF 20-2000
OSLO (2009) 20-2000
NAPLES (2009) 20-2000
NAPLES (2010) 20-2000
B
ABUNDANT
Planktonic size fraction
0.8-3 µm (pico)
3-20 µm (nano)
20-2000 µm (micro/meso)
RARE
Geographic location
BLANES
GIJON
NAPLES
OSLO
ROSCOFF
VARNA

Figure 3

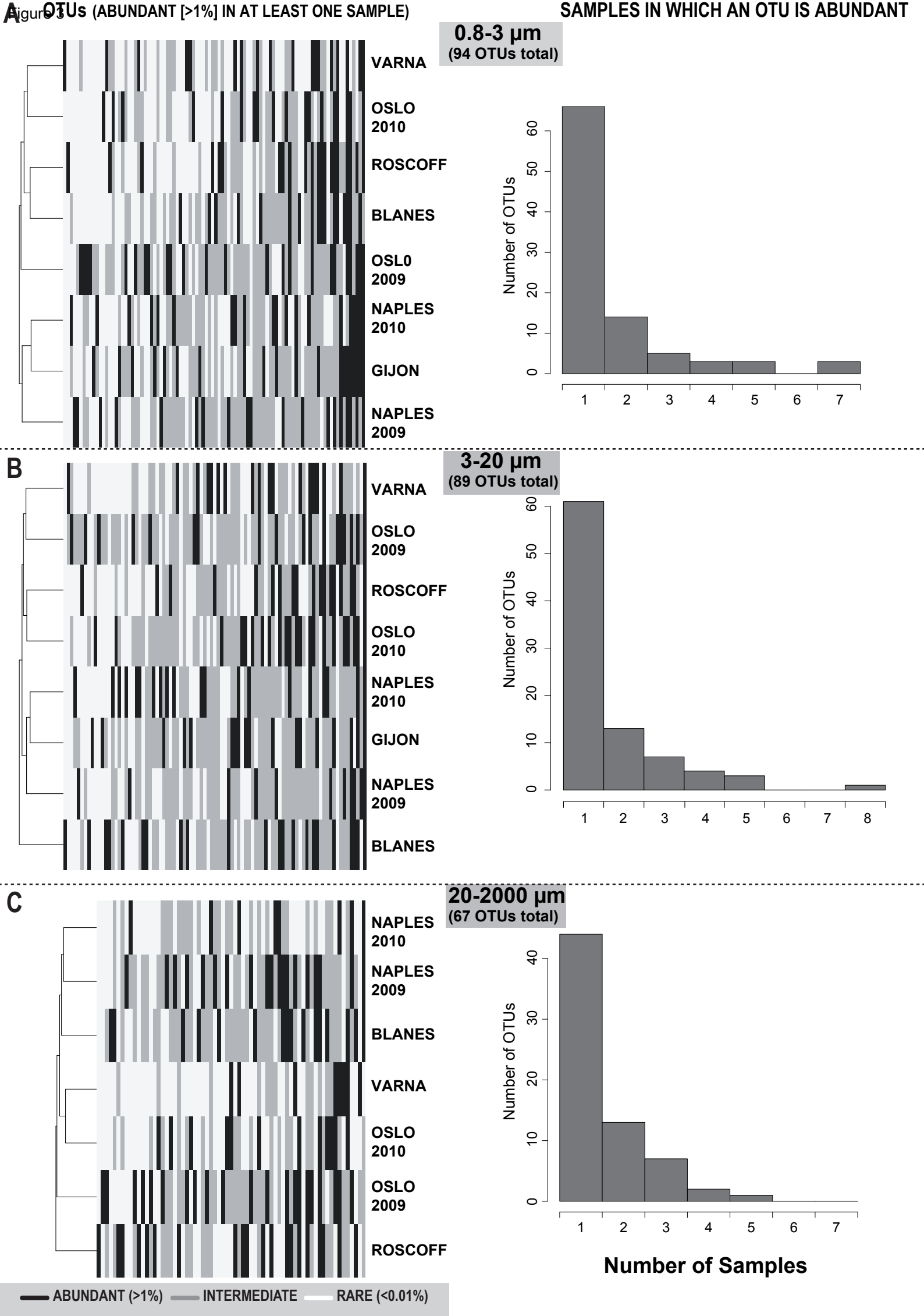
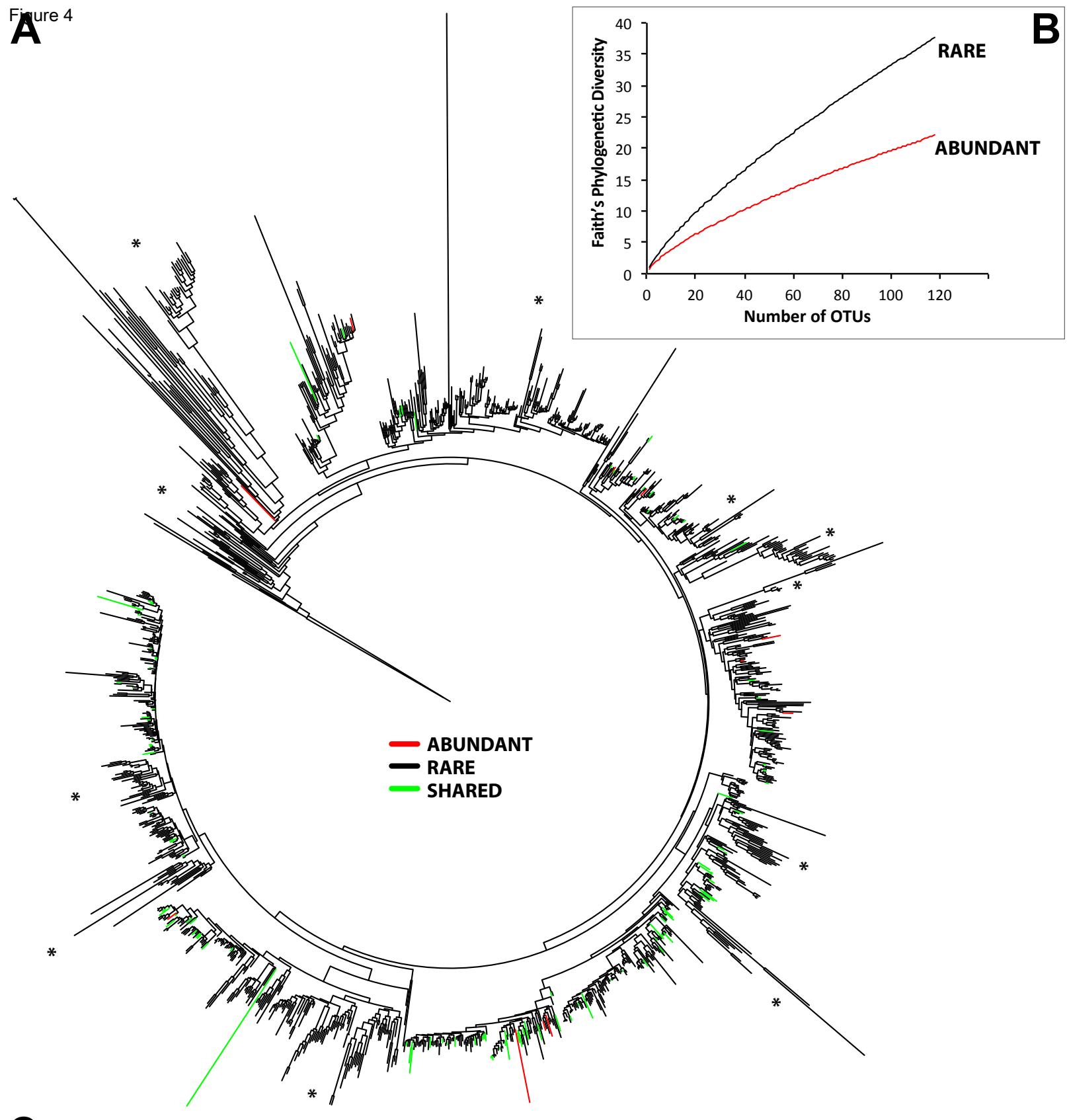

A
OTUs (ABUNDANT [>1%] IN AT LEAST ONE SAMPLE)
SAMPLES IN WHICH AN OTU IS ABUNDANT
0.8-3 µm
(94 OTUs total)
VARNA
OSLO 2010
ROSCOFF
BLANES
OSL0 2009
NAPLES 2010
GIJON
NAPLES 2009
Number of OTUs
B
3-20 µm
(89 OTUs total)
VARNA
OSLO 2009
ROSCOFF
OSLO 2010
NAPLES 2010
GIJON
NAPLES 2009
BLANES
Number of OTUs
C
20-2000 µm
(67 OTUs total)
NAPLES 2010
NAPLES 2009
BLANES
VARNA
OSLO 2010
OSLO 2009
ROSCOFF
Number of OTUs
Number of Samples
ABUNDANT (>1%)
INTERMEDIATE
RARE (<0.01%)

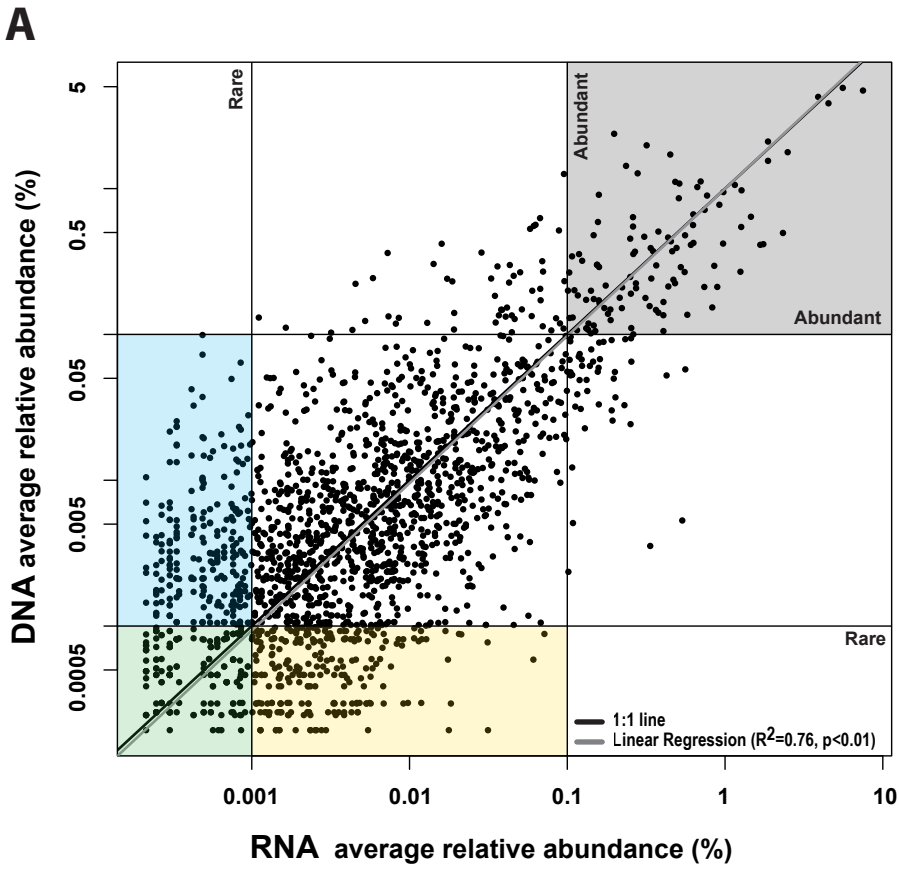
Figure 4



C

	# OTUs	$MPD_{observed}$	MPD_{null}	MPD_z	*p*	$MNTD_{observed}$	$MNTD_{null}$	$MNTD_z$	*p*
Regionally Abundant OTUs	118	0.94	1.25	-4.34	< 0.001	0.22	0.39	-4.62	< 0.001
Regionally Rare OTUs	1332	1.24	1.24	-0.1	0.386	0.19	0.19	-0.76	0.160

Figure 5


A
Rare
Abundant
Abundant
Rare
1:1 line
Linear Regression (R^2=0.76, p<0.01)
DNA average relative abundance (%)
0.0005
0.005
0.05
0.5
5
0.001
0.01
0.1
1
10
RNA average relative abundance (%)

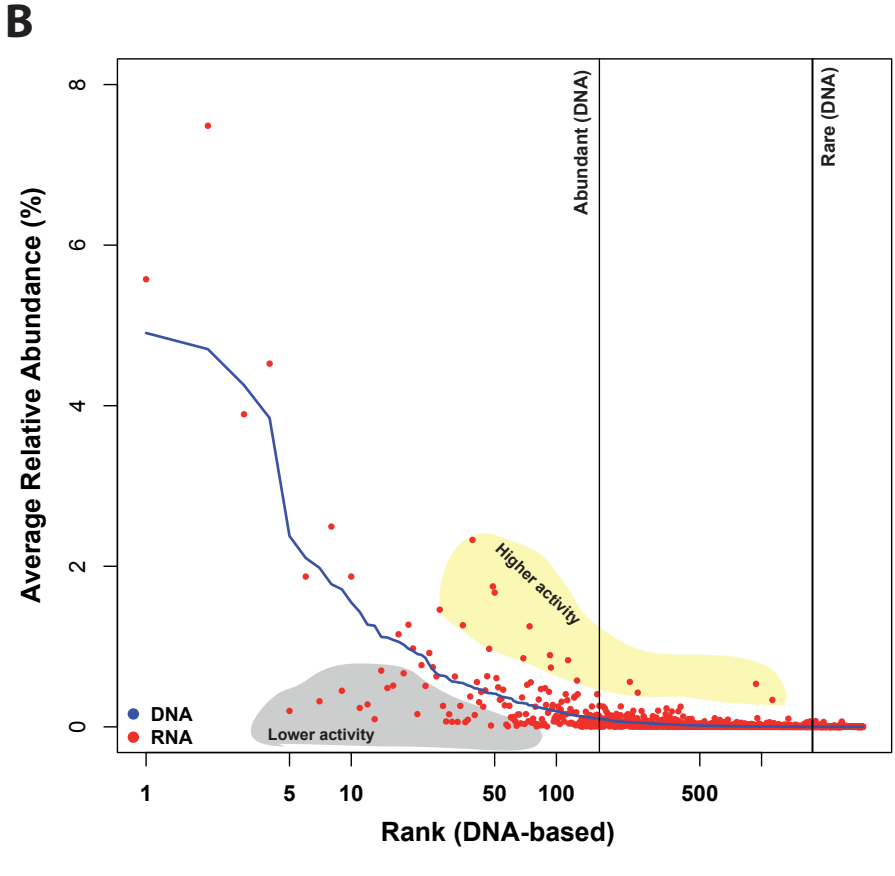

B
Abundant (DNA)
Rare (DNA)
Higher activity
Lower activity
DNA
RNA
Average Relative Abundance (%)
0
2
4
6
8
1
5
10
50
100
500
Rank (DNA-based)

Table 1. General description of both complete as well as normalized V9 18S rRNA *Illumina* datasets

	Combined size fractions	0.8-3 (µm)	3-20 (µm)	20-2000 (µm)
Number of clean reads	5696049 [a] / 1794000 [b]	2279669 [a] / 624000 [b]	2298280 [a] / 624000 [b]	1118100 [a] / 546000 [b]
Samples	23 / 23	8 / 8	8 / 8	7 / 7
Geographic sites	6 / 6	6 / 6	6 / 6	5 / 5
All OTUs [c]	9007 / 7035	6597 / 4412	7157 / 4786	3491 / 2941
Abundant OTUs [d]	155 (1.7%) / 154 (2.2%)	153 (2.3%) / 153 (3.5%)	143 (1.9%) / 144 (3.0%)	95 (2.7%) / 95 (3.2%)
Rare OTUs [e]	7333 (81%) / 5329 (75.7%)	5145 (77.9%) / 2981 (67.5%)	5614 (78.4%) / 3242 (67.7%)	2432 (69.6%) / 1865 (63.4%)

[a] All data; variable number of reads per sample
[b] Normalized data; 78000 reads per sample in all samples
[c] All OTUs included in the dataset
[d] OTUs abundant in the regional community; average relative abundances >0.1%
[e] OTUs rare in the regional community; average relative abundances <0.001%
See also Figures S1, S2, S3 & S5

Inventory of Supplemental Information

SUPPLEMENTAL DATA

Supplemental Figures

Figure S1: Distribution of all OTUs as well as regionally abundant and rare OTUs across the different organismal size fractions. Related to Figure 1, Table 1 & Experimental Procedures.

Figure S2: Rarefaction curves pointing to richness saturation. Related to Figure 1 & Table 1.

Figure S3: Regional Species (OTUs) Abundance Distributions (SADs) with different SAD models fitted, as well as regional SADs for the different organismal size fractions. Related to Table 1.

Figure S4: Species (OTUs) Abundance Distributions (SADs) with different SAD models fitted for each individual sample. Related to Figure 1.

Figure S5: Regional community data fitted to the Preston log-normal model and the associated calculation of the "veiled" OTUs. Related to Table 1.

Supplemental Tables

Table S1: General description of the community sequencing approach (using *Illumina* V9 18S rRNA) and the obtained OTUs (95 % clustering). Related to Figure 1 and Experimental Procedures.

Table S2: Comparison between 95% and 97% OTU clustering thresholds for the *Illumina* dataset. Related to Figure 1 and Results.

Table S3: Description of the sampling sites. Related to Figure 1 and Experimental Procedures.

Table S4: Description of samples where both the rDNA and rRNA were sequenced using *454*. Related to Figure 5 and Experimental Procedures.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Supplemental information on sampling, molecular methods and data analyses.

SUPPLEMENTAL REFERENCES

Includes all references mentioned in the Supplemental Information section.

SUPPLEMENTAL DATA

Supplemental Figures and Legends

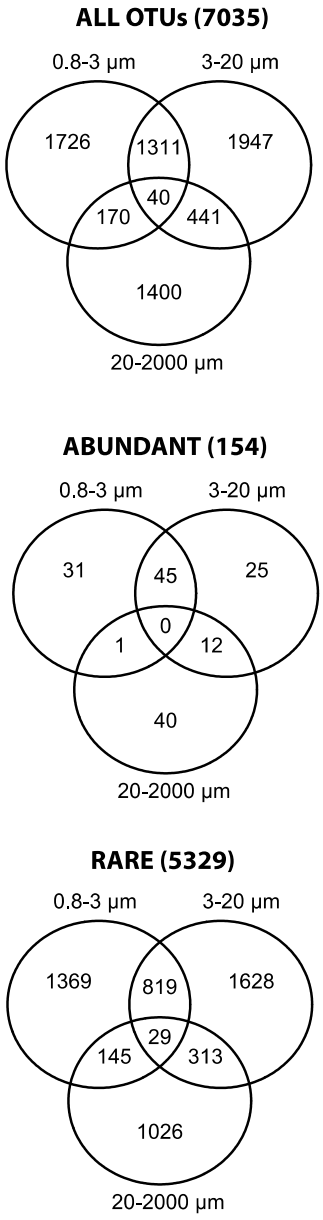


Figure S1 (related to Figure 1, Table 1 & Experimental Procedures).
Venn diagrams indicating the distribution of OTUs (normalized *Illumina* dataset) into the pico (0.8-3µm), nano (3-20µm), and micro/meso (20-2000µm) organismal size-fractions. The distribution is shown for all OTUs in the regional community (7035 OTUs), as well as for regionally abundant (154) and rare (5329) OTUs.

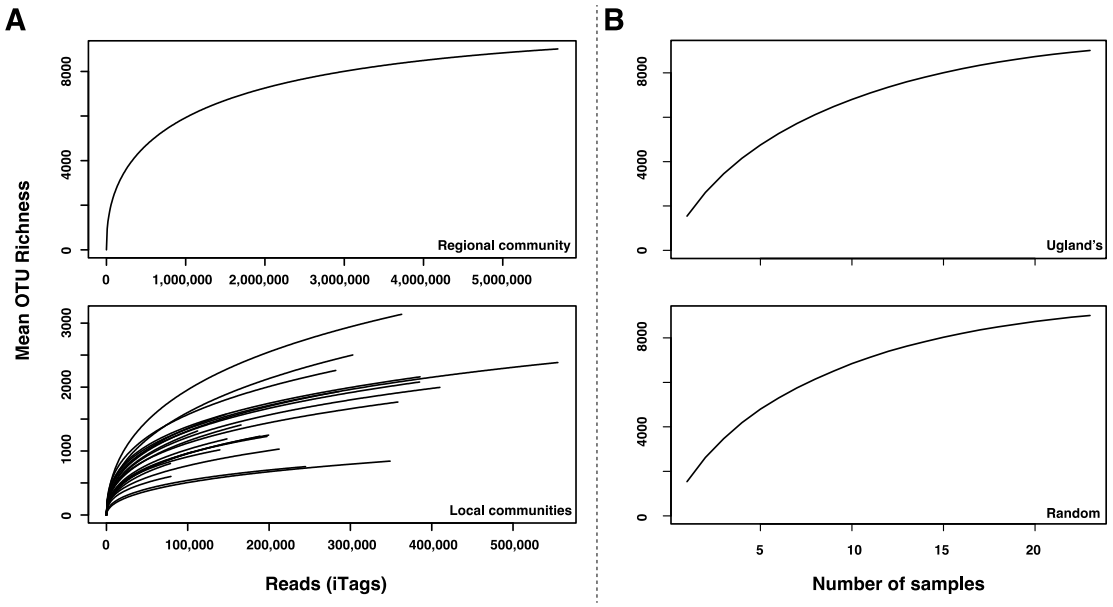


Figure S2 (related to Figure 1 & Table 1).
(A) Rarefaction curves using all *Illumina* tags for the combined set of samples (regional community; upper panel) as well as for the individual samples (local communities; lower panel). Note the different scales in y and x axes.
(B) Species (OTUs) accumulation curves based on the progressive addition of samples calculated using "Ugland" and "Random" methods.

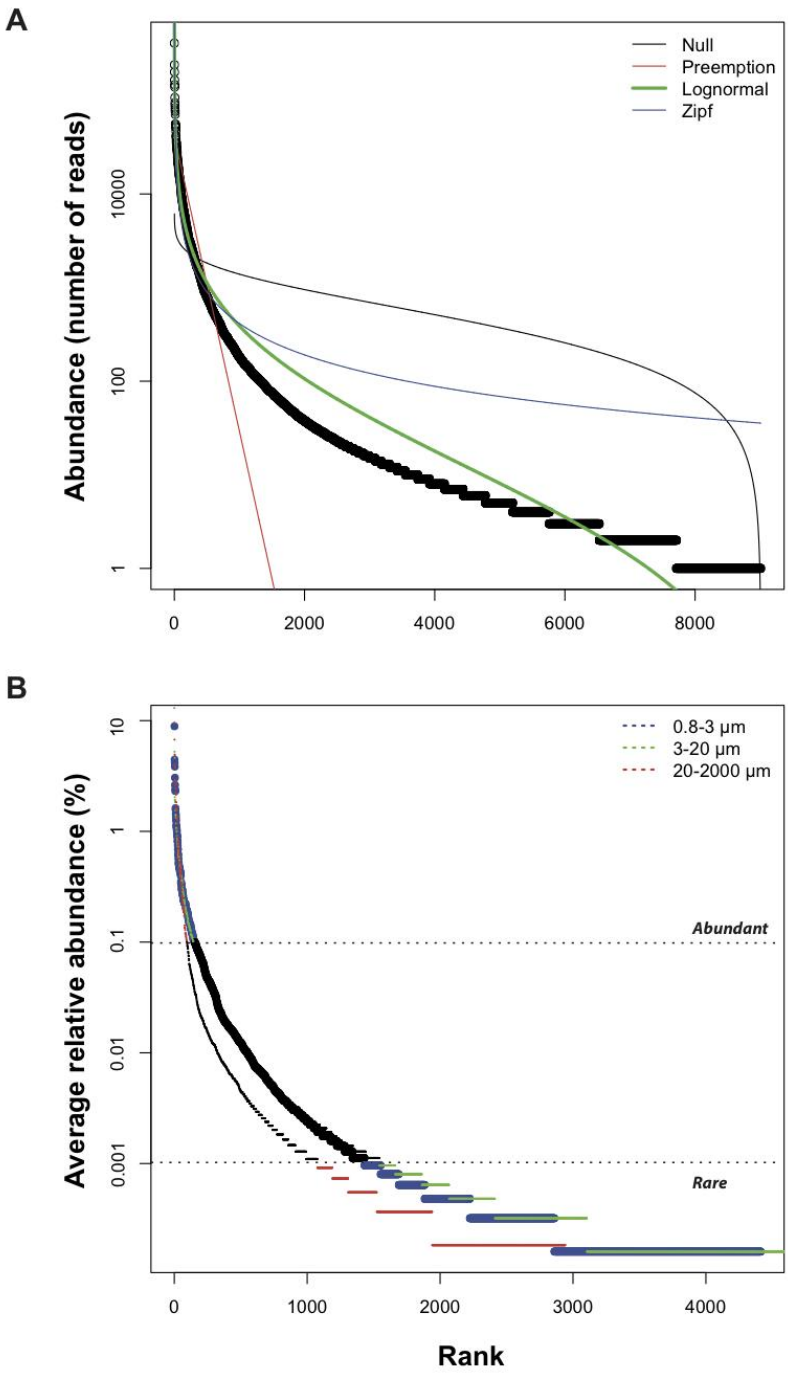


Figure S3 (related to Table 1).
(A) Species (OTUs) Abundance Distribution (SAD) for all pooled non-normalized samples (entire regional community) indicating the four fitted models (Null, Preemption, Lognormal & Zipf). The model with the best fit was the Lognormal according to the Akaike's Information Criterion.
(B) SADs for all normalized samples separated by organismal size fractions (size-fractionated regional community).

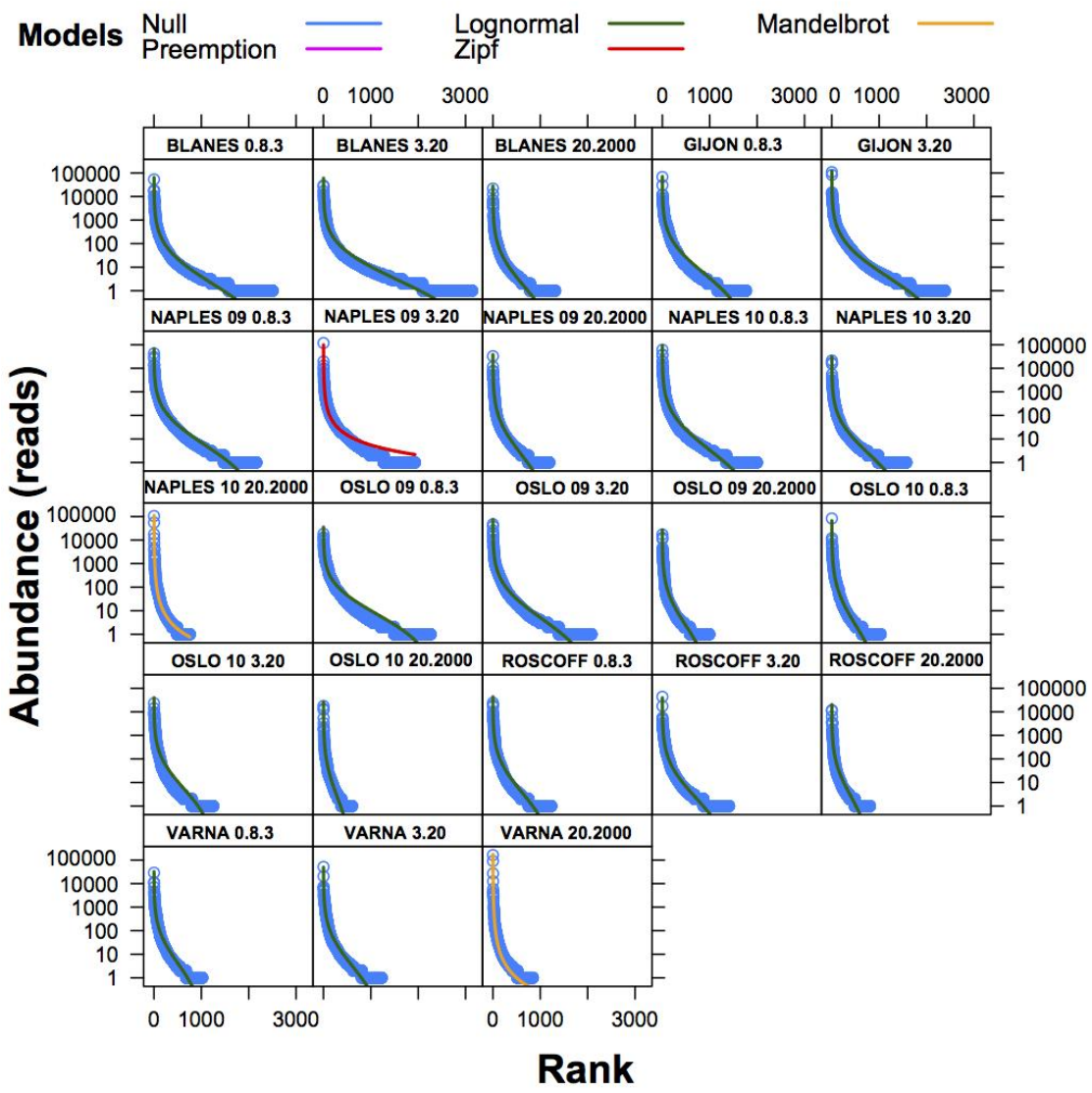


Figure S4 (related to Figure 1).
Species (OTUs) Abundance Distribution (SAD) for individual non-normalized samples. Five models were fitted to the SADs (Null, Preemption, Lognormal, Zipf & Zipf-Mandelbrot) and the model with the best fit according to the Akaike's Information Criterion is indicated for each sample with a colored curve. Note that in most samples, the log-normal model shows the best fit.

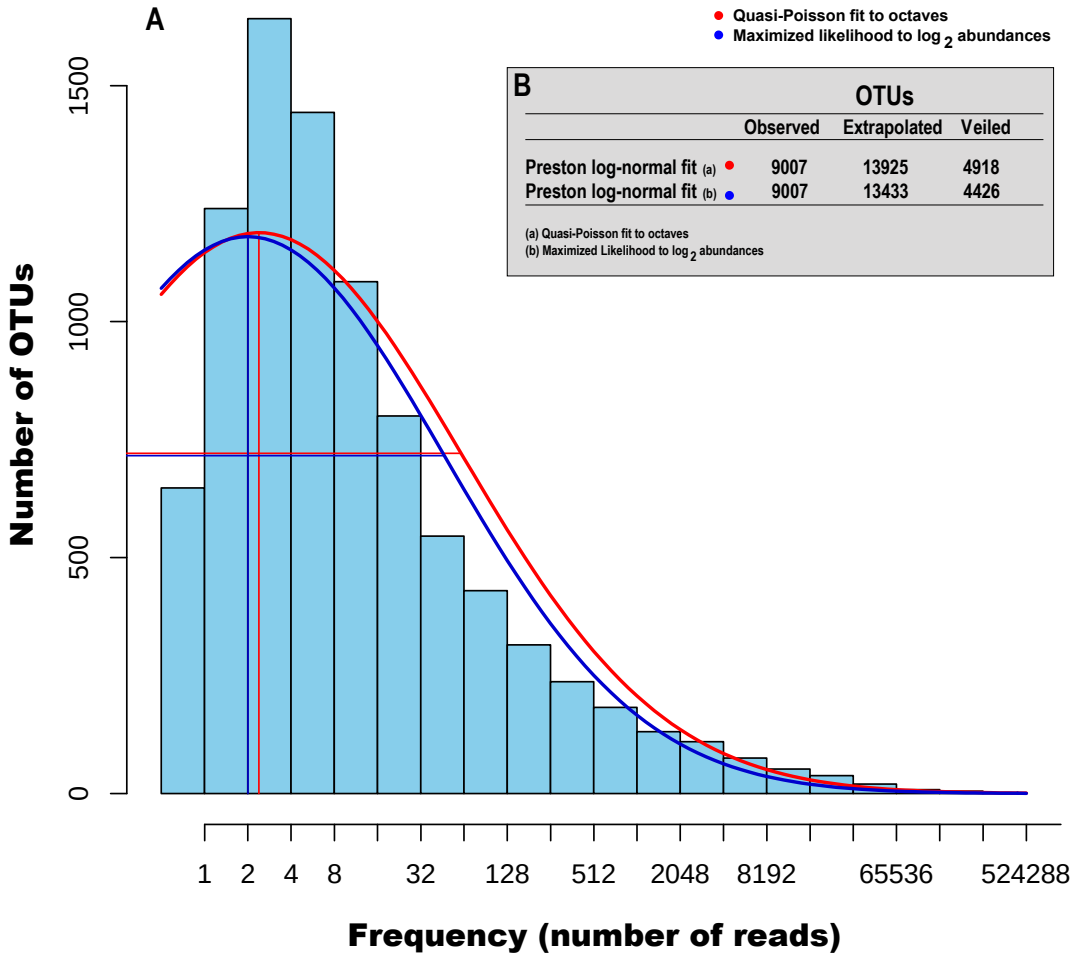


Figure S5 (related to Table 1).
(A) Fit of the entire dataset (regional community) to the Preston log-normal model using two approximations: Quasi-Poisson fit to octaves and Maximized likelihood to $\log_2$ abundances.
(B) Estimation of the "veiled" OTUs (i.e. OTUs not sampled) using both approximations presented in (A).

Supplemental Tables

Table S1 (related to Figure 1 & Experimental Procedures). General description of the community sequencing approach (using *Illumina* V9 18S rRNA reads; 90-100 bp) and the obtained OTUs (95 % clustering)

Sample #	Site (Country)	Year	Size fraction (µm)	# Raw reads [a]	# Clean reads [b]	% Clean reads	# OTUs (95%) [c]	Locally abundant OTUs (>1 %) [c]	Locally rare OTUs (<0.01%) [c]	% Locally abundant OTUs [c]	% Locally rare OTUs [c]
1*	Blanes (Spain)	2010	0.8-3	8089694	302912	3.7	1437	17	1082	1.2	75.3
2	Gijon (Spain)	2010	0.8-3	7554382	358386	4.7	1023	20	686	2.0	67.1
3	Naples (Italy)	2009	0.8-3	8431092	385651	4.6	1261	20	839	1.6	66.5
4	Naples (Italy)	2010	0.8-3	5858636	410095	7.0	1146	20	851	1.7	74.3
5*	Oslo (Norway)	2009	0.8-3	6009959	281955	4.7	1441	24	984	1.7	68.3
6*	Oslo (Norway)	2010	0.8-3	7154407	212433	3.0	701	19	475	2.7	67.8
7*	Roscoff (France)	2010	0.8-3	3544916	188820	5.3	857	22	604	2.6	70.5
8*	Varna (Bulgaria)	2010	0.8-3	8768061	139417	1.6	819	18	557	2.2	68.0
9*	Blanes (Spain)	2010	3-20	7120547	363060	5.1	1773	22	1359	1.2	76.6
10	Gijon (Spain)	2010	3-20	8425151	555166	6.6	1222	18	809	1.5	66.2
11	Naples (Italy)	2009	3-20	6023114	285961	4.7	1189	11	871	0.9	73.3
12*	Naples (Italy)	2010	3-20	5443795	146688	2.7	1230	20	911	1.6	74.1
13*	Oslo (Norway)	2009	3-20	6512071	385031	5.9	1215	18	830	1.5	68.3
14*	Oslo (Norway)	2010	3-20	5340317	199465	3.7	895	22	643	2.5	71.8
15	Roscoff (France)	2010	3-20	4432905	165498	3.7	1068	18	773	1.7	72.4
16*	Varna (Bulgaria)	2010	3-20	6060973	197411	3.3	894	18	616	2.0	68.9
17	Blanes (Spain)	2010	20-2000	9492667	112054	1.2	1138	17	838	1.5	73.6
18*	Naples (Italy)	2009	20-2000	7761710	148199	1.9	955	20	692	2.1	72.5
19*	Naples (Italy)	2010	20-2000	10087160	244797	2.4	492	8	358	1.6	72.8
20	Oslo (Norway)	2009	20-2000	4774982	106151	2.2	879	21	649	2.4	73.8
21*	Oslo (Norway)	2010	20-2000	6657899	79368	1.2	598	13	398	2.2	66.6
22*	Roscoff (France)	2010	20-2000	3196190	78643	2.5	803	20	596	2.5	74.2
23*	Varna (Bulgaria)	2010	20-2000	6770080	348888	5.2	468	8	350	1.7	74.8
	Sum			**153510708**	**5696049**	**N/A**				**N/A**	**N/A**
	Average			**6674378.6**	**247654.3**	**3.7**				**1.8**	**71.2**
	Standard deviation			**1787256.2**	**125008.1**	**1.7**				**0.5**	**3.3**

[a] Only the forward direction has been used.
[b] High-Quality reads remaining after a strict quality control: quality-checked reads with phred-average$_{(10bp\ window)}$ > 34 plus removal of chimeras, Bacteria, Archaea and Metazoa.
[c] Normalized OTUs using 78,000 reads in each sample.
* Samples that were sequenced also with *454* (rDNA/rRNA)

Table S2 (related to Figure 1 & Results). Comparison between 95% and 97% OTU clustering thresholds for the *Illumina* dataset. Number of OTUs and proportions of locally abundant or rare taxa are indicated

Site	Size fraction (µm)	Total OTUs (95%)[1]	Total OTUs (97%)[1]	#OTUs >1% (95%)[2]	#OTUs >1% (97%)[2]	%OTUs >1% (95%)[3]	%OTUs >1% (97%)[3]	#OTUs <0.01% (95%)[4]	#OTUs <0.01% (97%)[4]	%OTUs <0.01% (95%)[5]	%OTUs <0.01% (97%)[5]
Blanes	0.8-3	2502	3410	17	18	0.68	0.53	2166	2999	86.57	87.95
Gijon	0.8-3	1766	2555	20	20	1.13	0.78	1443	2181	81.71	85.36
Naples 2009	0.8-3	2160	2924	19	18	0.88	0.62	1754	2469	81.20	84.44
Naples 2010	0.8-3	1995	2925	19	18	0.95	0.62	1701	2569	85.26	87.83
Oslo 2009	0.8-3	2260	3364	24	21	1.06	0.62	1813	2834	80.22	84.24
Oslo 2010	0.8-3	1030	1416	18	15	1.75	1.06	816	1161	79.22	81.99
Roscoff	0.8-3	1231	1670	21	24	1.71	1.44	983	1390	79.85	83.23
Varna	0.8-3	1018	1480	17	18	1.67	1.22	748	1171	73.48	79.12
Blanes	3-20	3138	4167	22	20	0.70	0.48	2742	3674	87.38	88.17
Gijon	3-20	2384	3433	19	20	0.80	0.58	1973	2963	82.76	86.31
Naples 2009	3-20	1930	2629	11	14	0.57	0.53	1627	2265	84.30	86.15
Naples 2010	3-20	1572	2310	20	17	1.27	0.74	1260	1918	80.15	83.03
Oslo 2009	3-20	2079	3057	18	19	0.87	0.62	1706	2610	82.06	85.38
Oslo 2010	3-20	1249	1747	22	24	1.76	1.37	1006	1437	80.54	82.26
Roscoff	3-20	1407	1976	19	16	1.35	0.81	1117	1632	79.39	82.59
Varna	3-20	1228	1810	19	22	1.55	1.22	968	1507	78.83	83.26
Blanes	20-2000	1313	1722	16	17	1.22	0.99	1026	1315	78.14	76.36
Naples 2009	20-2000	1189	1616	19	18	1.60	1.11	940	1325	79.06	81.99
Naples 2010	20-2000	756	1090	9	8	1.19	0.73	623	930	82.41	85.32
Oslo 2009	20-2000	989	1356	21	18	2.12	1.33	768	1090	77.65	80.38
Oslo 2010	20-2000	603	876	12	13	1.99	1.48	403	631	66.83	72.03
Roscoff	20-2000	805	1082	20	23	2.48	2.13	597	827	74.16	76.43
Varna	20-2000	842	1507	9	8	1.07	0.53	734	1353	87.17	89.78
Average						1.32	0.94			80.36	83.20
Standard Dev.						0.50	0.42			4.66	4.23

[1] Total number of OTUs
[2] Number of locally abundant OTUs
[3] Percentage of locally abundant OTUs
[4] Number of locally rare OTUs
[5] Percentage of locally rare OTUs

Table S3 (related to Figure 1 & Experimental Procedures). Description of the sampling sites; all samples were taken from surface waters (<5m)

Description	Latitude , Longitude	Distance to coast (KM)	Max. depth[a] (m)	Sampling date	Temp. surface (°C)	Salinity surface	DCM (m)	ChlA[b] (µg/l)	Nitrate Surf./DCM[c] (µg/l)	Phosphate Surf./DCM (µg/l)	Tot-P Surf./DCM[d] (µg/l)
Blanes	41° 40' N, 2° 48' E	1.0	20	2/2010	12.5	37.5	N/A	1.0	2/2	7/6	13/13
Gijon	43° 40' N; 5° 35' W	12.0	110	9/2010	20.2	35.7	40	7.0	2/26	3/4	10/12
Naples	40° 48' N, 14° 15' E	4.0	75	10/2009	22.8	37.7	23	1.4	16/0	1/1	22/16
	40° 48' N, 14° 15' E	4.0	76	5/2010	19.2	37.2	35	1.2	<2/<2	4/3	14/8
Oslo	59° 16' N, 10° 43' E	1.5	100	09/2009	15.0	25.0	8	3.2	9/1	4/3	22/21
	59° 16' N, 10° 43' E	1.5	100	06/2010	15.0	22.0	9	1.9	<2/<2	3/2	12/11
Roscoff	48° 46' N, 3° 57' W	5.0	60	4/2010	9.9	34.9	ND	0.5	87/80	12/12	29/17
Varna	43°10' N, 28° 50' E	40.0	400	5/2010	21.5	16.0	40	8.0	2/2	4/3	11/11

Nutrients were measured according to Murphy and Riley [S1] and Grasshoff *et al.* [S2] in a Bran+Luebbe Autoanalyzer 3 at the University of Oslo. Note that Naples and Oslo have been sampled twice. N/A= not applicable, ND= no data.

[a] Maximum depth of the water column.

[b] Maximum Chlorophyll A concentration in the water column measured with fluorometry (fluorometer attached to a CTD).

[c] Values for Surface and DCM samples given in the format "Surface/DCM".

[d] Total Phosphorus.

Table S4 (related to Figure 5 & Experimental Procedures). Set of samples where both the rDNA and rRNA were sequenced using *454* (V4 18S)

Sample #	Site (Country)	Year	Size fraction (µm)	# Clean reads RNA[1]	# Clean reads DNA[1]
1	Blanes (Spain)	2010	0.8-3	13679	8210
2	Oslo (Norway)	2009	0.8-3	22061	14419
3	Oslo (Norway)	2010	0.8-3	31172	27197
4	Roscoff (France)	2010	0.8-3	7055	12639
5	Varna (Bulgaria)	2010	0.8-3	7938	7691
6	Blanes (Spain)	2010	3-20	19951	6268
7	Naples (Italy)	2010	3-20	12257	8474
8	Oslo (Norway)	2009	3-20	26883	26009
9	Oslo (Norway)	2010	3-20	7921	34514
10	Varna (Bulgaria)	2010	3-20	24868	22444
11	Naples (Italy)	2009	20-2000	8750	22655
12	Naples (Italy)	2010	20-2000	16058	3158
13	Oslo (Norway)	2010	20-2000	11747	5487
14	Roscoff (France)	2010	20-2000	3498	6553
15	Varna (Bulgaria)	2010	20-2000	19247	16180
Total				233085	221898

[1] Total number of *454* reads after quality control and denoising (DeNoiser). A total of 454,983 reads were used for both RNA and DNA.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Sampling and RNA/DNA extraction

Surface (<5m) seawater samples were collected through the BioMarKs consortium (http://www.BioMarKs.org/) in six European coastal stations: offshore Blanes (Mediterranean, Spain), Gijon (Bay of Biscay, Spain), Naples (Mediterranean, Italy), Oslo (North Sea / Skagerrak, Norway), Roscoff (English Channel, France), and Varna (Black Sea, Bulgaria) (see Figure 1, Table S3). Pico- (0.8-3µm) and nano- (3-20µm) plankton samples were collected using Niskin bottles. A total of 15 to 40 liters of water were pre-filtered through a 20µm sieve and then sequentially filtered through a polycarbonate membrane of 3 and 0.8 µm. Meso/micro- (20-2000µm) plankton samples were collected and concentrated using a 20µm-porosity plankton net during 20 min, then pre-filtered through a 2,000µm sieve and afterwards filtered through a 20µm polycarbonate membrane. Filtration time did not surpass 30 minutes to avoid RNA degradation. Filters were flash-frozen and stored at -80° C. Total DNA and RNA were extracted simultaneously from the same filter using the NucleoSpin® RNA L kit (Macherey-Nagel) and quantified using a Nanodrop ND-1000 Spectrophotometer. To remove DNA from RNA extracts, we used the TurboDNA kit (Ambion). A total of 100ng of extracted RNA were immediately reverse transcribed to DNA using the RT Superscript III random primers kit (Invitrogen).

Amplification and Illumina-sequencing of the V9 18S rRNA

The hypervariable V9 18S region was amplified with the eukaryotic primers 1389f (5'-TTGTACACACCGCCC-3') and 1510r (5'-CCTTCYGCAGGTTCACCTAC-3') [S3]. The V9 was chosen as it normally has between 90-140bp in microeukaryotes [S3], and

therefore most of it can be covered by 100bp *Illumina* GAIIx reads. Amplifications were conducted with Phusion® High-Fidelity DNA Polymerase (Finnzymes). The PCR mixture (25µL final volume) contained about 5ng of cDNA template with 0.35µM final concentration of each primer, 3% of DMSO and 2X of GC buffer Phusion Master Mix (Finnzymes). Amplifications were done following the PCR program: initial denaturation step at 98°C for 30 sec, followed by 25 cycles of 10 sec at 98°C, 30 sec at 57°C, 30 sec at 72°C, and a final elongation step at 72°C for 10 minutes. Each sample was amplified in triplicates to get enough concentration of amplicons and reduce potential biases. Products of the reactions were run on 1.5% agarose gels to check for successful amplification within the expected sequence length. Amplicons were then pooled and purified using the NucleoSpin® Extract II kit (Macherey-Nagel). Ligation of amplicons with *Illumina* adapters and library construction were performed according to *Illumina* instructions. Paired-end 100bp sequencing was performed using a Genome Analyzer IIx (GAIIx) system (*Illumina*) located at Genoscope (http://www.genoscope.cns.fr/spip/, France).

Amplification and 454-sequencing of the V4 18S rDNA/rRNA

The universal primers TAReuk454FWD1 (5'-CCAGCASCYGCGGTAATTCC-3') and TAReukREV3 (5'-ACTTTCGTTCTTGATYRA-3') were used to amplify the hypervariable V4 region (~380 bp) of the eukaryotic 18S rDNA/rRNA [S4]. The primers were adapted for *454* using the manufacturers specifications for Lib-L unidirectional sequencing, and had the configuration A-adapter-Tag (7 or 8bp)- forward primer, and B-adapter-reverse primer. PCR reactions were performed in 25µl, and consisted in 1X MasterMix Phusion® High-Fidelity DNA Polymerase (Finnzymes), 0.35µM of each primer, and 3% DMSO. We added a total of 5ng of template

DNA/cDNA to each PCR reaction. PCR reactions consisted of an initial denaturation step at 98°C during 30 sec, followed by 10 cycles of 10 sec at 98°C, 30 sec at 53°C and 30 sec at 72°C, and afterwards by 15 cycles of 10 sec at 98°C, 30 sec at 48°C and 30 sec at 72°C, including a final elongation step at 72°C for 10 minutes. Amplicons were checked in 1.5% agarose gels for successful amplification within the expected length. Triplicate amplicon reactions were pooled and purified using NucleoSpin® Extract II (Macherey-Nagel). Purified amplicons were eluted in 30µl of elution buffer and quantified using Quant-it dsDNA Picogreen kit (Invitrogen). The final total amount of pooled amplicons for *454* tag-sequencing was approximately 1µg. Amplicon sequencing was carried out on a *454* GS FLX Titanium system (*454* Life Sciences, USA) installed at Genoscope (http://www.genoscope.cns.fr/spip/, France).

Sequence analysis for Illumina reads

A total of 23 samples sequenced with *Illumina* GAIIx and based on RNA (cDNA) template were used (Table S1). We used RNA as this molecule is much less stable than DNA in extracellular conditions. Therefore, most RNA recovered from the environment should have originated from living cells (ensuring that nucleic acids originate from living cells is particularly relevant when investigating the rare biosphere). Furthermore, there are studies indicating that RNA provides a better representation of community composition than DNA in microbial eukaryotes (less biases due to uneven rDNA copy number between taxa) [S5].

Despite using paired-end reads (2x100bp), we opted for using the forward reads only, as some merged (assembled) reads presented reduced quality; similar strategies have been used in other works [S6]. *Illumina* GAIIx fastq files (Phred +64) were translated into different fasta and phred-quality files. The number of raw (i.e. not quality

checked) reads obtained per sample is indicated in Table S1. Reads were quality-checked using a sliding window (window length = 10bp) and each window had to have a phred-quality average >34 to pass the control (see [S7]). Quality-checked reads had a minimum length of 90bp with a maximum homopolymer size = 10bp; no ambiguous bases were allowed. This highly stringent cleaning protocol insured only reads of the highest quality were retained for downstream analysis. All quality checks were run in Mothur v1.2X [S8]. The number of clean reads after quality control is shown in Table S1. Quality-checked reads were analyzed in QIIME v1.4 [S9]. Reads were clustered into OTUs using UCLUST v1.2.22 with a 95% similarity threshold and the parameters --max_accepts =20 and --max_rejects =500. This conservative clustering threshold was selected in order to reduce any artifactual increase in richness produced by sequencing errors that could remain in our dataset (see [S10]). Chimeras were detected using ChimeraSlayer [S11] with a reference database derived from PR2 [S12], and subsequently removed. One representative sequence per OTU (the most abundant) was selected. OTU representative sequences were assigned taxonomy by BLASTing them (blastn; [S13]) against the databases SILVA v108 (BLAST threshold e-value=e^{-6}) as well as a PR2-derived database [S12] (BLAST threshold e-value=e^{-100}). Both the SILVA v108 and the PR2-derived reference databases were pre-clustered at 97% similarity to reduce the chances of ambiguous classifications (hereafter referred as SILVA $v108_{(97\%)}$ & $PR2_{(97\%)}$). The initial BLAST against SILVA $v108_{(97\%)}$ served to detect and remove unwanted taxa (e.g. Bacteria, Archaea and Metazoa). Further OTU removal was done using the classifications obtained with the $PR2_{(97\%)}$. The final curated *Illumina* RNA dataset included 5,696,049 reads.

Sequence Analysis for 454 reads

A total of 15 samples, where both DNA and RNA (cDNA) were sequenced using *454* (V4 18S), were used in downstream analyses (Table S4). Note that these samples were all included in the main *Illumina* dataset (Table S1). All *454* reads were run through QIIME v1.4. Only reads between 200-500bp were used. Reads were checked for quality using a sliding window of 50bp (Phred average >25 in each window), truncated to the last good window and subsequently checked again for minimum length. Reads that passed the quality control were denoised using DeNoiser (v 0.851; [S14]) as implemented in QIIME v1.4. Subsequently, reads were clustered into OTUs using UCLUST v1.2.22 with a 99% threshold of sequence similarity (compared to *Illumina* reads, a higher clustering threshold was used since this region is longer, less hypervariable and furthermore, this dataset was denoised). UCLUST was run with the parameters --max_accepts =20 and --max_rejects =500. One representative read per OTU (the most abundant) was selected. Chimeras were detected using ChimeraSlayer with a reference database derived from PR2 [S12], and subsequently removed. Representative reads were assigned taxonomy by BLASTing them against the databases SILVA v108 $_{(97\%)}$ (BLAST threshold e-value=e^{-6}), the custom PR2$_{(97\%)}$ (BLAST threshold e-value=e^{-100}), as well as the MP database (BLAST threshold e-value=e^{-100}). MP is an in-house 18S V4 database that contains only marine microeukaryotes, with improved taxonomy for specific groups [S15]. The MP database was also pre-clustered at 97% to prevent ambiguous classifications. Thus, each database provided different degrees of taxonomic resolution. After taxonomy assignment, Metazoan sequences were removed (Bacteria and Archaea were not present). In the final dataset, after all unwanted sequences were removed, RNA included 233,085 reads and DNA 221,898 reads (total 454,983 reads).

Construction of OTU tables

Single singletons as well as OTUs present in only one sample were removed from the global dataset (i.e. the dataset including samples that were not considered in our work, such as deeper planktonic or sediment samples). After the exclusion of samples that were not considered in our analyses, single singletons were present again in both the *Illumina* and *454* datasets, but these were included in downstream analyses as they were already validated by other reads in unused samples.

Diversity and network analyses

Most analyses were run in the R [S16] environment. Venn diagrams were calculated using the R-package Limma [S17]. Rarefactions as well as Species (OTUs) Accumulation Curves (SAC) were calculated using the R-package Vegan [S18]. SACs were estimated using the methods "Ugland" and "Random". Ugland's method finds the expected SAC using the method proposed by Ugland et al. [S19]. The "Random" method calculates the mean SAC from random permutations of the data [S20]. Rank-abundance (or Species Abundance Distribution) plots were produced in R and different models were fitted using the "radfit" function in Vegan. The fitted models were "Null" (brokenstick), "Preemption" (niche preemption model [geometric series] or Motomura model), "Lognormal" (log-normal model), "Zipf" (Zipf model) and "Mandelbrot" (Zipf-Mandelbrot model) (see [S21]); the "Mandelbrot" model could not be fitted to all datasets. In addition, we fitted our data to the Preston log-normal model [S22] using the functions "prestonfit" (fit estimation using second degree log-polynomial with Poisson error) and "prestondistr" (fit estimation using direct maximization of log-likelihood) in Vegan (see Vegan user manual for more details). OTU networks were constructed in

QIIME using the subsampled OTU table and graphically edited in Cytoscape [S23] using the layout "Edge-Weighted Spring Embedded" with eweights. Networks were filtered using eweight values (eweight thresholds: "Abundant"=500, "Rare"=3, "All"=200).

Mapping of short Illumina reads to longer reference sequences and phylogeny construction

Regionally abundant or rare OTUs (one representative read per OTU) were mapped using BLASTn to a V9 18S rDNA Sanger reference database based on the custom $PR2_{(97\%)}$ database. We did not BLAST OTUs against the custom PR2 database containing areas other than V9 as this produced poorer results. To construct the reference phylogeny, we used the full-length 18S sequences corresponding to the entire custom V9 $PR2_{(97\%)}$ database. Only sequences >1,500bp were used for phylogeny construction, leaving a dataset of 8,311 reference sequences. Sequences were aligned using Mothur against the aligned SILVA 108 (eukaryotes only). A Maximum Likelihood tree (FULL-TREE) was inferred using RAxML HPC-MPI (v7.2.8; [S24]) under the model GTR+CAT/G+I and checked against other phylogenies of marine protists [S15]. The FULL-TREE was pruned using the R-package APE (Analyses of Phylogenetics and Evolution; [S25]) to keep only those reference taxa that were hit by abundant or rare OTUs (PRUNED-TREE). The PRUNED-TREE had 1,343 Sanger sequences (the tree had less taxa than the sum of rare and abundant OTUs [5,483] since we allowed for the mapping of multiple OTUs to the same reference taxa). The PRUNED-TREE and the corresponding annotation were uploaded to iTol [S26] for graphical representation. In addition, the PRUNED-TREE was used for the calculation of phylogenetic metrics. The Mean Phylogenetic Distance (MPD) and Mean Nearest

Taxon Distance (MNTD) [S27] were calculated using the R-package Picante [S28] and compared to a null model that shuffled taxa-labels 1,000 times across all taxa included in the phylogeny-based distance matrix (see [S29]).

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